

## CLAIMS

1. A vector comprising:
  - (a). two or more genes encoding sugar-nucleotide regenerating enzymes selected from the group consisting of GalK, GalT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, PgM, NagE, Agm1, glmU, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and
  - (b). one or more genes encoding glycosyltransferase(s),wherein said genes are operably linked to a promoter.
2. The vector of claim 1 comprising genes encoding three or more enzymes for regenerating a sugar-nucleotide.
3. The vector of claim 1 comprising genes encoding two or more glycosyltransferases.
4. The vector of claim 1 comprising genes encoding three or more glycosyltransferases.
5. The vector of claim 1 comprising genes encoding GalK, GalT, and GalU.
6. The vector of claim 5 further comprising a gene encoding Ndk.
7. The vector of claim 5 further comprising a gene encoding Ppk.
8. The vector of claim 5 further comprising a gene encoding PykF.
9. The vector of claim 5 further comprising genes encoding PoxB, Ndk, and Ppa.
10. The vector of claim 1 comprising a gene encoding SusA.



11. The vector of claim 10 further comprising a gene encoding GalE.
12. The vector of claim 10 further comprising a gene encoding GluT.
13. The vector of claim 10 further comprising genes encoding Ugd and UGT2B7.
14. The vector of claim 1, wherein the one or more glycosyltransferase(s) is selected from the group consisting of a galactosyltransferase, a glucosyltransferase, an N-acetylglucosaminyl transferase, an N-acetylgalactosaminyl transferase, a glucuronyltransferase, a sialyltransferase, a mannosyltransferase, and a fucosyltransferase.
15. The vector of claim 14, wherein the galactosyltransferase is selected from the group consisting of LgtB and LgtC.
16. The vector of claim 14, wherein the glucosyltransferase is selected from the group consisting of LgtF, Alg5, and DUGT.
17. The vector of claim 14, wherein the N-acetylglucosaminyl transferase is LgtA.
18. The vector of claim 14, wherein the N-acetylgalactosaminyl transferase is UDP-GalNAc:2'-fucosylgalactoside- $\alpha$ -3-N-acetylgalactosaminyl transferase.
19. The vector of claim 14, wherein the glucuronyltransferase is UGT2B7.
20. The vector of claim 14, wherein the sialyltransferase is SiaT 0160.
21. The vector of claim 14, wherein the mannosyltransferase is selected from the group consisting of Alg1 and Alg2.
22. The vector of claim 14, wherein the fucosyltransferase is selected from the group consisting of  $\alpha$ 1,3-FucT,  $\alpha$ 1,2-FucT, and  $\alpha$ 1,3/4-FucT.



23. The vector of claim 1 wherein the promoter is an inducible promoter.
24. The vector of claim 23, wherein the inducible promoter is  $\lambda$  P<sub>R</sub> promoter.
25. The vector of claim 24 further comprising a  $\lambda$  C<sub>I</sub> repressor gene.
26. The vector of claim 1, wherein at least one gene is operably linked to a ribosomal binding site sequence.
27. The vector of claim 26, wherein each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a ribosomal binding site sequence.
28. The vector of claim 1, wherein at least one gene is operably linked to an IRES.
29. The vector of claim 1, wherein at least one gene is operably linked to a tag sequence.
30. The vector of claim 29, wherein each gene encoding a sugar-nucleotide regenerating enzyme or a glycosyltransferase is operably linked to a tag sequence.
31. The vector of claim 29, wherein the tag sequence encodes polyhistidine.
32. The vector of claim 1, wherein the vector encodes an epimerase.
33. The vector of claim 1, wherein the vector encodes a fusion protein.
34. The vector of claim 33, wherein the fusion protein comprises an epimerase and a glycosyltransferase.
35. The vector of claim 34, wherein the epimerase is UDP-Gal-4-epimerase.



36. The vector of claim 35, wherein the glycosyltransferase is  $\alpha$ -1,3-galactosyltransferase.
37. The vector of claims 1, wherein the vector is selected from the group consisting of plasmids, phage, phagemids, viruses, and artificial chromosomes.
- 5 38. The vector of claim 37, wherein the vector is a plasmid.
39. A cell comprising heterologous genes encoding one or more sugar-nucleotide regenerating enzyme and one or more glycosyltransferase.
40. The cell of claim 39, wherein the cell is a prokaryotic cell.
41. The cell of claim 40, wherein the prokaryotic cell is a bacterium.
- 10 42. The cell of claim 41, wherein the bacterium is *E. coli*.
43. The cell of claim 42, wherein the *E. coli* is LacZ.
44. The cell of claim 39, wherein the cell is a eukaryotic cell.
45. The cell of claim 44, wherein the eukaryotic cell is a yeast.
46. The cell of claim 39, wherein at least one of the heterologous genes is integrated into the genome of the cell.
- 15 47. The cell of claim 39, wherein the heterologous genes are encoded within one or more plasmids.
48. The cell of claim 47, wherein the heterologous genes are encoded within one plasmid.
- 20 49. A method of producing a glycoconjugate comprising the step of contacting a cell comprising heterologous genes encoding:



(i). one or more encoding sugar-nucleotide regenerating enzymes selected from the group consisting of GalK, GalT, GalU, PykF, Ndk, PpK, AcK, PoxB, Ppa, PgM, NagE, Agm1, glmU, a GalNAc kinase, a pyrophosphorylase, Ugd, NanA, Cmk, NeuA, Alg2, Alg1, SusA, ManB, ManC, a phosphomannomutase, GalE, GMP, GMD, and GFS; and

(ii). one or more glycosyltransferase,

with a bioenergetic.

50. A kit comprising the plasmid of claim 1.

51. A non-human cell comprising the plasmid of claim 1.